Figure 6. Lovastatin concentration from the corresponding wild-type *lovE* control is shown in matching fill pattern. For example, *lovE* alleles 2, 7, 8 and 9 were all transformed and assayed at the same time as the non-hatched wild-type control. The horizontal line in each individual box represents the median.

Lovastatin concentration was also determined by high pressure liquid chromatography (HPLC). Briefly, 100 µL of broth sample was removed and diluted 1:10 into 70% H_2O-30% acetonitrile (900 µl). This mixture was spun down to pellet debris at 13000 RPM for 5 minutes. 900 µl of this diluted broth was transferred to a vial and the sample was analyzed by HPLC. 10 µl were injected into a Waters HPLC system (996 photo-diode array detector, 600 E pump controller and 717 autosampler) equipped with a YMC-Pack ODS column (Aq-302-3, 150 x 4.6 mm ID, S-3 µM pore size) and eluted with isocratic 40% aqueous acetic acid (0.7%)-60% acetonitrile for 8 minutes. Lovastatin was detected at 238 nm to have a retention time of 6.5 minutes and was quantified using a calibration curve created from pure lovastatin samples.

The results from ten individual transformants for each *lovE* variant are shown in standard box plot format in Figure 7A and 7B. Thirty individual wild-type *lovE* transformants and ten individual MB2143 negative control transformants were tested. Identical controls are plotted in Figures 7A and 7B.

PCR analysis of *A. terreus* transformants demonstrates that greater than fifty percent of the transformants contain the transgene. Variability in levels of transgene expression can presumably be influenced by integration site and copy number. *lovE* variants containing identical amino acid substitutions are labeled.

The amino acid and nucleic acid sequences of *lovE* variant sequences are presented in Table 5 and Table 6, respectively.